*DOI:*
10.1021/acs.jnatprod.6b00610

*Document Version*
Peer reviewed version

# Cytotoxic Naphthoquinone and Azaanthraquinone Derivatives from an Endophytic *Fusarium solani*

Nargis Sultana Chowdhury,[†‡ §] Md. Hossain Sohrab,[†*] Md. Sohel Rana,[§] Choudhury Mahmood Hasan,[⊥] Shirin Jamshidi,[Δ] Khondaker Miraz Rahman[Δ *]

† *Pharmaceutical Sciences Research Division (PSRD), BCSIR Laboratories, Dhaka, Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka, Bangladesh*

‡*Department of Pharmacy, Manarat International University, Dhaka, Bangladesh*

**§** *Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh*

⊥*Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka, Bangladesh*

**Δ** *Institute of Pharmaceutical Sciences, King's College London, 7 Trinity Street, London SE1 1DB*

## ABSTRACT

Bioactivity guided fractionation of the ethyl acetate extract obtained from the culture of the endophytic fungus *Fusarium solani* resulted in the isolation of one new naphthoquinone 9-desmethylherbarine (**1**), and two azaanthraquinone derivatives, 7-desmethylscorpinone (**2**) and 7-desmethyl-6-methylbostrycoidin (**3**), along with four known compounds. Their structures were elucidated by spectral analysis, as well as a direct comparison of spectral data with those of known compounds. Azaanthraquinones **2** and **3** showed cytotoxic activity against four human tumour cell lines, MDA MB 231, MIA PaCa2, HeLa and NCI H1975. A molecular docking study suggested DNA interactions as the mode of action of these naphthoquinones and azaanthraquinones.

Endophytes are microorganisms that inhabit the interior of plants (especially leaves, stems and roots) and show no apparent harm to their host.[1] Recently, endophytes are being viewed as an outstanding source of secondary metabolites. These microorganisms received considerable attention in the last 20 years when their capacity to protect against insect and pest pathogens was noticed.[2] They have proven to be promising sources of new biologically active natural products of interest for specific medicinal or agrochemical applications.[3]

Naphthoquinone metabolites are widespread in nature and show a wide range of biological activities. A considerable number of naphthoquinones have also been isolated from fungi. Species of the genus *Fusarium* produce a wide variety of metabolites, e.g., fusarubin,[4] anhydrofusarubin,[5] javanicin,[6] fusariumin,[7] fusarimine,[8] fusarone[9] and cyclic lipopeptide,[10] which exhibit antimicrobial, cytotoxic[11] and phytotoxic[12] activities.

In the continuation of our search for bioactive secondary metabolites from endophytic fungi, a *Fusarium solani* strain, isolated from the plant *Aponogeton undulatus* was investigated (Table 1). Chemical investigation of the ethyl acetate extract of the endophytic fungus led to the isolation of three new compounds, namely 9-desmethylherbarine (**1**), 7-desmethylscorpinone (**2**) and 7-desmethyl-6-methylbostrycoidin (**3**), along with four known compounds, fusarubin (**4**), anhydrofusarubin (**5**), javanicin (**6**)[13] and cerevesterol (**7**)[14] (Figure 1). The known metabolites were identified by comparison of their spectroscopic data with those reported in the literature. We report herein the isolation and structural elucidation of the metabolites, the cytotoxic activities of the new naphthoquinone **1,** azaanthraquinones **2** and **3**, and a molecular modelling study to understand their mode of action and elucidate their mode of interaction with DNA.

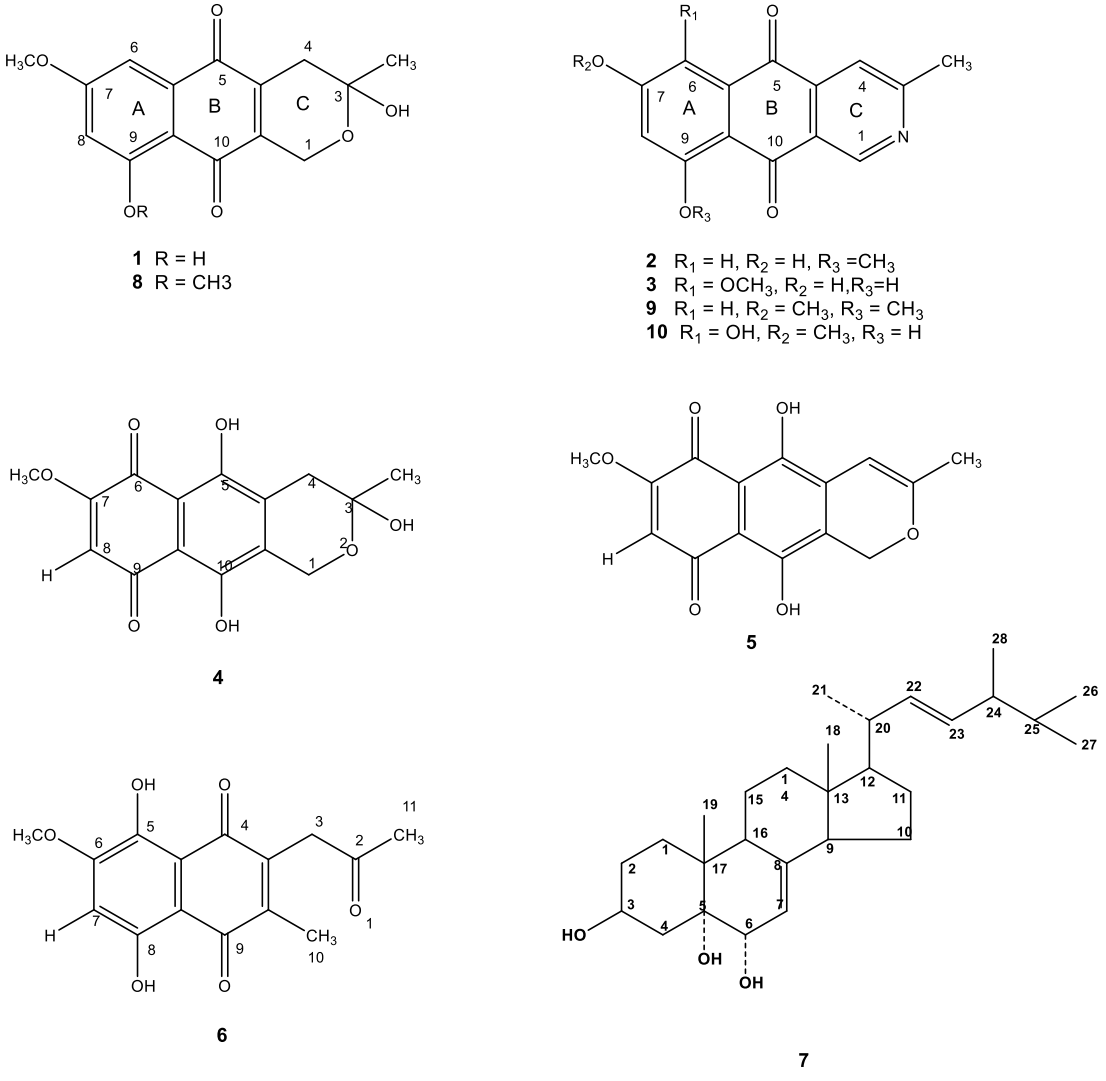


**Figure 1**. Structures of the isolated compounds

A total of 4 (four) endophytic fungi, namely AULE-1, AURE-1, AURE-3 and AURE-4 were isolated and purified from the plant *Aponogeton undulatus*. The endophytic fungus *Fusarium solani* (internal strain no. AURE-4) was selected for further investigation, based on the brine shrimp lethality bioassay data (ESI), and was cultured at a large scale to isolate bioactive secondary metabolites. The ethyl acetate extract of *Fusarium solani* grown in potato dextrose agar media was subjected to repeated column and preparative thin layer chromatography to yield three new compounds (**1, 2** and **3**) as well as four known compounds (**4, 5 , 6** and **7**). Compound **1** is a naphthoquinone derivative, and compounds with a chemical scaffold similar to **2** and **3** have been referred to as azaanthraquinones. Compounds **4, 5** and **6** were previously reported from the fungus *Fusarium solani*[13] whereas **7** was obtained for the first

time from the genus *Fusarium* sp. This is the first report of isolation of the metabolites **1 - 7** from an endophytic fungus of the plant *Aponogeton undulatus* Roxb.

**Table 1**. $^{1}H$ (400 and 700 MHz, $CDCl_3$) and $^{13}C$ (100 MHz and 176 MHz, $CDCl_3$) NMR Spectroscopic Data for Compounds **1-3**

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| position | $\delta_C$, type | $\delta_H$, (*J* in Hz) | $\delta_C$, type | $\delta_H$, (*J* in Hz) | $\delta_C$, type | $\delta_H$, (*J* in Hz) |
| 1 | 57.8, $CH_2$ | 4.72, s | 150.5, CH | 9.43, s | 138.7, CH | 9.51, s |
| 2 | - | - | - | - | - | - |
| 3 | 94.5, C | - | 164.8, C | - | 165.4, C | - |
| 3-$CH_3$ | 29.4, $CH_3$ | 1.61, s | 25.3, $CH_3$ | 2.77, s | 25.3, $CH_3$ | 2.81, s |
| 3-OH | - | 2.25, s | - | - | - | - |
| 4 | 32.0, $CH_2$ | 2.86, d (19.2)<br>2.53, d (19.2) | 117.8, CH | 7.87,s | 118.0, CH | 7.97, s |
| 4a | 141.5, C | - | 125.9, C | - | 138.7, C | - |
| 5 | 182.8, C | - | 183.2, C | - | 183.7, C | - |
| 5a | 140.1, C | - | 133.3, C | - | 123.5, C | - |
| 6 | 106.0, CH | 7.17,d (2.0) | 108.2, CH | 7.36, d (2.4) | 149.3, CH | - |
| 6-$OCH_3$ | - | - | - | - | 56.8, $CH_3$ | 4.04, s |
| 7 | 164.4, C | - | 150.5, C | - | 149.3, C | - |
| 7-OH | - | - | - | 5.10, s | - | 13.51, s |
| 7-$OCH_3$ | 56.1, $CH_3$ | 3.89, s | - | - | - | - |
| 8 | 109.2, CH | 6.61,d (2.0) | 107.4 , CH | 6.76, d (2.4) | 107.9, CH | 6.77, s |
| 9 | 166.0, C | - | 142.6, C | | 161.3, C | - |
| 9-OH | - | 12.11, s | - | - | - | 8.12, s |
| 9-$OCH_3$ | - | - | 56.1, $CH_3$ | 3.94, s | - | - |
| 9a | 109.1, C | - | 118.5, C | - | 118.1, C | - |
| 10 | 186.5, C | - | 182.8, C | - | 183.7, C | - |
| 10a | 141.5, C | - | 136.4, C | - | 124.6, C | - |

Compound **1** was obtained as an orange amorphous powder; HR-ESIMS in conjunction with NMR data suggested a molecular formula of $C_{15}H_{14}O_6$. The $^1$H NMR spectrum showed the presence of a benzene ring with two *meta*-coupled aromatic protons ($\delta$ 7.17 and $\delta$ 6.61). A one proton sharp singlet at $\delta$ 12.11 could be attributed to a phenolic chelated hydroxy group. Both $^1$H NMR and $^{13}$C NMR data of **1**, with some difference, were close to that of herbarin (**8**).[15] In the latter compound there are two methoxyl groups ($\delta_H$ 3.94 and $\delta_H$ 3.95; $\delta_C$ 56.11 and $\delta_C$ 56.55), whereas, compound **1** showed the signal of one methoxyl group ($\delta_H$ 3.89; $\delta_C$ 56.1). The presence of one methoxyl group and one phenolic chelated OH group in **1** indicated the replacement of the methoxyl group at C-9 of herbarin (**8**) with a hydroxy group in **1**. The relative configuration of **1** was established by NOESY interactions. The protons of the C-3 methyl group exhibited (Figure 2) nearly equipotent NOESY correlations to both protons of the neighboring methylene group (4a-H and 4b-H), so it should be in an equatorial position.[15] This data supports that compound **1** is 9-desmethylherbarine, a new naphthoquinone derivative.

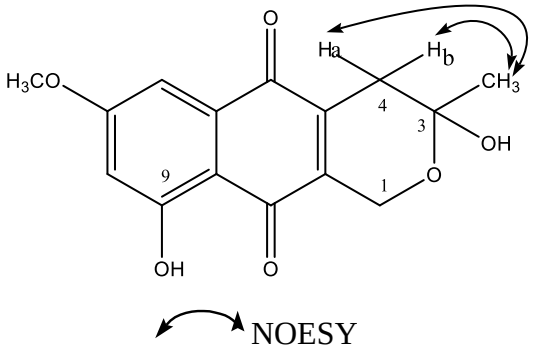


**Figure 2**. Relative structure of **1**

Compound **2** was obtained as a yellow amorphous powder and a molecular formula of $C_{15}H_{11}O_4N$ was suggested from its HR-ESIMS and NMR data. Although both $^1$H NMR and $^{13}$C NMR data of **2** were in close correspondence to those of scorpinone (**9**),[16] some differences were noted. Scorpinone (**9**) has got two methoxyl funtions ($\delta_H$ 3.86 and $\delta_H$ 3.53;

$\delta_C$ 56.1 and $\delta_C$ 56.6), on the other hand **2** showed the presence of one methoxyl group ($\delta_H$ 3.94; $\delta_C$ 56.1). The presence of a one proton singlet at $\delta$ 5.10 ppm in $^1$H NMR could be attributed to one phenolic non-chelated OH group in **2**. The loss of one methoxyl group along with the presence of one phenolic non-chelated OH group in **2**, compared to scorpinone (**9**), confirmed the placement of the hydroxy group at C-7 of **2**. Thus, compound **2** was identified as 7-desmethylscorpinone, a new azaanthraquinone.

Compound **3** was obtained as a red amorphous powder. The HR-ESIMS data along with the NMR data suggested a molecular formula of $C_{15}H_{11}O_5N$. $^1$H NMR and $^{13}$C NMR data of compound **3** indicated it, with slight differences, to be structurally related to bostrycoidin (**10**).[17] There are two 1H sharp singlets at $\delta$ 13.20 and $\delta$ 13.49 ppm in the $^1$H NMR of bostrycoidin (**10**) due to the two phenolic chelated hydroxy groups. There is only one signal for phenolic chelated hydroxy group at $\delta$ 13.51 ppm that could be observed in **3**. Moreover, the 1H proton resonance at $\delta$ 8.12 proved the presence of a non-chelated OH group in **3**. A three proton singlet at $\delta_H$ 4.04 and $\delta_C$ 56.8 revealed the presence of a methoxyl group in **3** as in bostrycoidin (**10**). The position of the methoxyl group in **3** will be at either C-6 or C-9. This was confirmed by the mass spectrum with a molecular ion base peak at *m/z* 286.0707 without any dehydrated fragments. This data supports that compound **3** is a new 2-azaanthraquinone, 7-desmethyl-6-methylbostrycoidin.

Compound **3** is the C-6 methoxylated and C-9 demethylated derivative of compound **2**. The differences in the $^{13}$C chemical shifts values observed for compound **2** and **3** prompted us to carry out computational experiments to rationalize the observed values. The electronic density surfaces of the two compounds (Figure S2) showed considerable differences, and this can potentially explain the differences in the observed chemical shifts. The remaining isolated metabolites were identified as fusarubin (**4**), anhydrofusarubin (**5**), javanicin (**6**) and cerevesterol (**7**) (Figure 1) by comparison of their spectral data with those reported for these compounds.

The cytotoxicity of new compounds **1 - 3** was determined against four human tumor cell lines including HeLa cervical carcinoma, MDA MB 231 breast cancer, MIA PaCa2 pancreatic cancer and NCI H1975 non-small cell lung cancer; doxorubicin was used as the positive control. Compounds **2** and **3** were active against all four cell lines (Table 2) with low micromolar to sub-micromolar $IC_{50}$ , while **1** was weakly active. The dose response curves of

**1 - 3** and the control compound doxorubicin against the triple negative breast cancer cell line MDA MB 231 are shown in Figure S4. It can be seen from the $IC_{50}$ values (Table 2) that the activities of **2** and **3** were comparable, but **3** showed most potent activity. Compound **2** showed sub-micromolar $IC_{50}$s against three cell lines and low micromolar $IC_{50}$s against MDA-MB 231 cell line. Naphthoquinone **1** which has a non-aromatic dihydropyran ring (ring C) was notably less active compared to the azaanthraquinones **2** and **3** that have an aromatic pyridine ring (ring C) and are noticeably planar. The compounds were tested against the non-tumor cell line WI-38 to determine their selectivity and explore their potential as anticancer compounds.

**Table 2: Cytotoxicity Assay Results of compounds 1 – 3 against Human Tumor Cells ($IC_{50}$ in µM)**

| Compound | HeLa (Cervical) | MDA MB 231 (Breast) | MIA PaCa 2 (Pancreatic) | NCI HI975 (Non-small cell lung) | WI38 (Human lung fibroblast) |
|---|---|---|---|---|---|
| **1** | 10.30 | 30.72 | 20.46 | 27.73 | 54.34 |
| **2** | 0.96 | 1.51 | 0.98 | 0.61 | 5.84 |
| **3** | 0.71 | 0.73 | 0.64 | 0.34 | 6.42 |
| **Doxorubicin** | 0.05 | 0.07 | 0.04 | 0.03 | 0.35 |

Naphthoquinones are known to work by intercalating DNA and **2** and **3** have planar structures that can similarly intercalate DNA. On the other hand, the ring C of **1** produces a kink in the structure and may prevent intercalation by sterically interacting with the DNA bases. Therefore, we expected **2** and **3** to intercalate DNA more efficiently compared to **1**, and this may explain the differences in cytotoxicity against the tumor cell lines tested. To rationalise this observation, a molecular modelling study was carried out with a mixed AT/GC 17-mer DNA sequence 5'-TAGCTAGCTAGCTAGCG-3', and all three ligands were docked to the DNA structure to elucidate their mode of interaction with DNA (Figure 3).

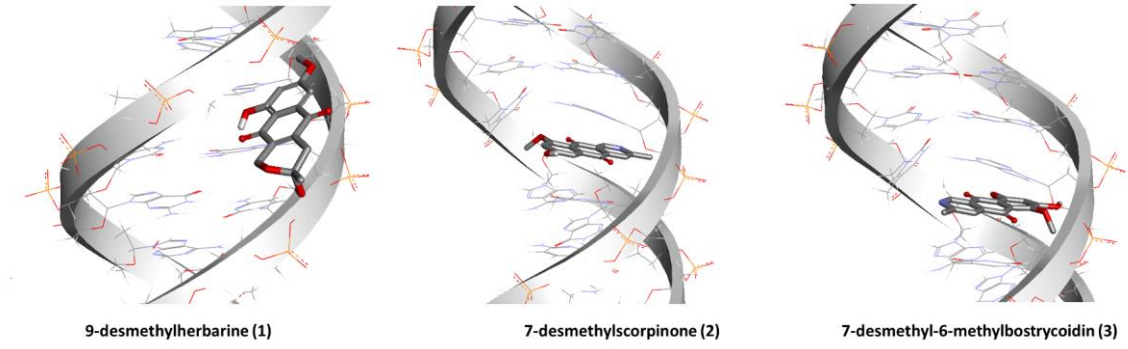


**Figure 3:** Mode of interaction of **1**, **2** and **3** after SMINA molecular docking with mixed AT/GC DNA sequence 5'-TAGCTAGCTAGCTAGCG-3' (key interaction sequences have been zoomed in, see the ESI for full images).

It can be seen from the Figure 3 that **2** and **3** can efficiently intercalate between the DNA base pairs and form stable complexes with a free energy of binding -8.3 kcal / mole and -7.9 kcal / mole, respectively. On the other hand, the ring C of compound **1** creates a steric clash with the DNA base pairs during intercalation and the intercalation complex is unfavorable with a free energy of binding 5.9 kcal / mole, and the compound formed a favourable minor groove complex with -7.8 Kcal / mole free energy of binding. The differences in the planarity of **2** and **3** compared to **1** were further confirmed by measuring the torsion angles of the rings after the molecular docking experiment. The torsional angle between ring B and ring C of **1** was notably lower, 173.18°, compared to the torsional angle between ring B and ring C of **2** and **3** which were calculated as 179.8° and 176.98°, respectively (Figure 4).

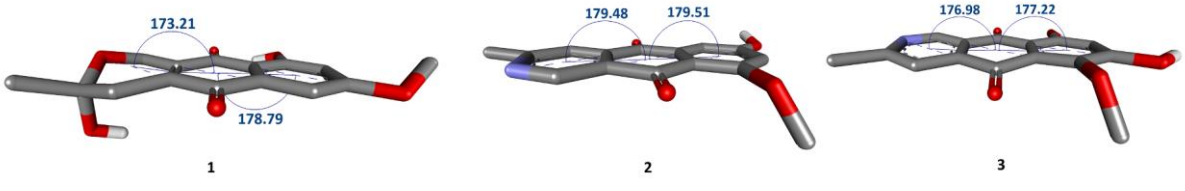


**Figure 4:** Torsional angles of the rings of **1 - 3.** The values were measured after the molecular docking of the compounds with the mixed AT / GC sequence.

The findings of the molecular modelling study support superior cytotoxicity observed for azaanthraquinones **2** and **3** compared to naphthoquinone **1**. Further mechanistic evaluation is required to confirm the linkage between the abilities of these molecules to intercalate DNA and their toxicity against the tumor cell lines.

## Experimental Section

### General Experimental Procedures

The NMR spectra were recorded on a Bruker 400 and 700 MHz NMR spectrometer using $CDCl_3$. The HRMS spectra were recorded on an Exactive Orbitrap by a Thermo Scientific Mass Spectrometer at King's College London, *(*London, UK) and the data was processed by Thermo XCalibur 2.2.

Infrared spectra (IR) spectra were measured on a SHIMADZUIRPrestige-21 MIR spectrometer. Ultraviolet (UV) spectra were obtained on a SPECORD 250 PLUS UV-VIS spectrophotometer using methanol. Column chromatography (CC) was carried out on silica gel (70-230 and 230-400 mesh, Merck, Germany). Organic solvents, potato dextrose agar medium and TLC plates were purchased from Merck, Germany.

### Fungal material

The fungus *Fusarium solani* (internal strain no. AURE-4, Genbank accession number KY511422) was isolated from fresh healthy roots of *Aponogeton undulatus* Roxb. growing in the water. The plant samples were collected from Chalanbil area of Natore district, Rajshahi, Bangladesh in October, 2013. The plant material was identified and authenticated by Dr. Sardar Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium (BNH). A voucher specimen of this collection is maintained at BNH under the accession number DACB – 32072 (ESI). About 300 grams of fresh and healthy parts of the plant (leaves, root, and nodule) were cut with a sterile scalpel and stored at 4 ºC in a sterile polythene bag prior to use. Endophytic fungi were isolated from the fresh plant parts following the procedure, established at Pharmaceutical Sciences Research Division, BCSIR Laboratories, Dhaka, Bangladesh.[18]

## Identification of the Endophytic Fungus

The endophytic fungus was grown on PDA for 28 days at 28 °C and morphologically characterized (ESI). The mycelium was scraped directly from the surface of the agar culture (5 days old) and weighed. Nucleic acid was extracted and purified using the DNA isolation kit for genomic DNA (Maxwell 16, Promega, USA) using the manufacturer protocol. For identification and differentiation, the Internal Transcript Spacer regions (ITS4 and ITS5) and the intervening 5.8S rRNA region was amplified and sequenced using electrophoretic sequencing on an ABI 3730 x l DNA analyzer (Applied Biosystems, USA) using Big Dye Terminator v 3.1 cycle sequencing kit. Finally, the sequence data (ESI) was deposited to Genbank database (accession number KY511422). The sequence data revealed 99% similarity to another fungal isolate of accession number KC764913.1 that has shown 99% similarity with *Fusarium solani* (accession numbers KM235740.1, KJ207395.1, KJ719812.1, FJ719812.1, EU625403.1, KU382502.1 etc.) deposited in the U.S National Center for Biotechnology Information (NCBI).

## Extraction and isolation

*Fusarium solani* (internal strain no.AURE-4), which had been isolated following surface sterilization from the roots of the plant *Aponogeton undulatus* Roxb. was cultivated at 28º C for 28 days on potato dextrose agar. After the completion of the incubation, the culture media were extracted three times with ethyl acetate to obtain the crude extracts. The extracts of the fungi were filtered and concentrated at low temperature and reduced pressure.[19] The crude extract (4.3 g) was subjected to column chromatography for fractionation on silica gel by using gradients of petroleum ether-ethyl acetate, then ethyl acetate, followed by a gradient of ethyl acetate-methanol, and finally methanol to afford a total of 15 fractions. These fractions were screened by TLC on silica gel under UV light and by spraying with vanillin - $H_2SO_4$ spray reagents. The column fraction of petroleum ether - 15% ethyl acetate was subjected to preparative TLC on silica gel (toluene - 5% EtOAc, 3 developments) to obtain **1** (6 mg, orange amorphous powder), **2** (5 mg, yellow amorphous powder) and **5** (18 mg, violet solid). The column fraction of petroleum ether - 20% ethyl acetate was subjected to preparative TLC on silica gel (toluene - 10% EtOAc, 2 developments) to obtain **6** (3 mg, red

amorphous powder). Two slightly impure red solid masses obtained from the column fractions of petroleum ether - 35% EtOAc and petroleum ether - 95% EtOAc were further purified by washing with different solvents to give **3** (7 mg, red amorphous powder) and **4** ( 80 mg, red solid), respectively. Adopting a similar procedure, crude crystals of the polar fraction of EtOAc - 5% MeOH were purified to give **7** (80 mg, white needle).The purity of compounds **1**, **2** and **3** was analysed by HPLC, and each of them was found to be at least 85% pure (ESI).

***9-desmethylherbarine (1)***

Orange amorphous powder; UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 220 (4.02), 265 (3.68), 267 (3.68) nm; IR (KBr): $\nu_{max}$ = 3552, 3473, 3414, 2360, 1637, 1616 $cm^{-1}$., HR-ESIMS $[M + Na]^+$*m/z* 313.0680 (calculated for $C_{15}H_{14}O_6Na$, 313.0680,); Purity – 87%, $^{13}C$ and $^1H$ data, see Table 1.

***7-desmethylscorpinone (2)***

Yellow amorphous powder; UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 230 (3.94), 232 (3.93), 416 (3.83) nm; IR (KBr): $\nu_{max}$ = 3552, 3475, 3414, 3234, 1637, 1618 $cm^{-1}$; HR-ESIMS $[M + H]^+$*m/z* 270.0758 (calculated for $C_{15}H_{11}O_4N$, 270.0761); Purity – 85%, $^{13}C$(176 MHz, $CDCl_3$) and$^1H$ data, see Table 1.

***7-desmethyl-6-methylbostrycoidin (3)***

Red amorphous powder; UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 253 (3.69), 314 (3.18), 528 (3.15) nm; IR (KBr): $\nu_{max}$ = 3564, 3552, 3473, 3414, 1618 cm-$^1$; HR-ESIMS $[M + H]^+$*m/z* 286.0707 (calculated for $C_{15}H_{12}O_5N$, 286.0710); Purity – 92%, $^{13}C$ and $^1H$ data, see Table 1.

The spectral data for compounds 4-7 can be found in the ESI document.

**Cell culture**

Compounds were tested for their cytotoxic activity against four immortalised human tumor cell lines; the human cervical cancer cell line HeLa, triple negative human breast cancer cell line MDA MB231, human pancreatic adenocarcinoma cell line MIA PaCa 2 and human non-small cell lung cancer cell line NCI H1975 were obtained from the American

Type Culture Collection and LGC Standards. All cell-lines were maintained in a monolayer culture in 75 $cm^2$ flasks (TPP, Switzerland) under a humidified 5% $CO_2$ atmosphere at 37 °C. The HeLa cell line was maintained in Dulbecco's Modified Eagles Media (DMEM; Invitrogen), supplemented with foetal bovine serum (10% v/v; Invitrogen), L-glutamine (2 mM; Invitrogen), non-essential amino acids (1x; Invitrogen) and penicillin-streptomycin (1% v/v, Invitrogen). For MDA MB231, high glucose DMEM (4.5g\l; Invitrogen), foetal bovine serum (10%, Biosera UK), non-essential amino acids (1x; Invitrogen), L-glutamine (2mM; Invitrogen) and penicillin-streptomycin (1% v/v, Invitrogen) was used for sub-culturing. For MIA PaCa2, Dulbecco's MEM, supplemented with L-glutamine (2mM; Invitrogen) and foetal calf serum (10%, Biosera UK) was used. The NCI H1975 cell lines were maintained by Roswell Park Memorial Institute Medium 1640 (RPMI 1640, Invitrogen) supplemented with foetal bovine serum (10% v/v; Invitrogen) and penicillin-streptomycin (1% v/v, Invitrogen). For WI 38 cell line, Dulbecco's MEM, supplemented with L-glutamine (2mM; Invitrogen), non-essential amino acids (1x; Invitrogen), penicillin-streptomycin (1% v/v, Invitrogen) and foetal bovine serum (15%, Biosera UK) was used. For passaging, cells were washed with PBS (GIBCO 14040, Invitrogen, UK), incubated with trypsin (GIBCO 25300, Invitrogen, UK), and re-seeded into fresh media. For seeding, cells were counted using a Neubauer haemocytometer (Assistant, Germany) by microscopy (Nikon, USA) on a non-adherent suspension of cells that were washed in PBS, trypsinised, centrifuged at 4 °C at 4000 rpm for 5 min and re-suspended in fresh media.

**MTT Assay**

The cells were grown in normal cell culture conditions at 37 ºC under a 5% $CO_2$ humidified atmosphere using an appropriate medium. The cell count was adjusted to 105 cells/mL and 5,000-20,000 cells were added per well depending on the cell line. The cells were incubated for 24 hours and 1 μL of the appropriate inhibitor concentrations were added to the wells in triplicates. After 96 h of continuous exposure to each compound, the cytotoxicity was determined using the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Lancaster Synthesis Ltd, UK) colorimetric assay. Absorbance was quantified by spectrophotometry at $\lambda$ = 570 nm (Envision Plate Reader,

PerkinElmer, USA). $IC_{50}$ values were calculated by a dose-response analysis using the Prism GraphPad Prism® software.

**Computational study**

Docking protocols are used in order to predict the binding affinities and binding sites of ligands into the biomacromolecules including DNAs and proteins. The scoring functions method of the docking program evaluates the accuracy of the docking procedure. Based on the fitness function scores and ligand binding position, the best-docked poses for each ligand are selected. Double Strand (DS) DNAs type B (BDNA) with 17 base pairs was generated by the NAB module of AMBER 12.0 package program, using 5'-TAGCTAGCTAGCTAGCG-3' sequence. PDB files for the ligands were generated by ChemDraw 15.0.

All the DNA and ligands structures were optimised by SYBYL software before molecular docking. Molecular docking of the ligands into the generated DNA sequence was performed by SMINA molecular docking program.[20] Blind molecular docking was run for finding the best binding site of ligands in the generated DNA among all the probable modes of binding in the DNA, including minor and major grooves and intercalation mode. All parameters were kept at their default values. In order to run blind molecular docking, the grid box was prepared to cover the full length of DNA to allow the ligand molecules to explore all the possible binding modes. For providing the intercalation mode for ligands, a gap opening between stacked base pairs is required. For this purpose, anthracene, which is very similar to the ligands, has been applied.[21] Presence of anthracene between the base pairs in the DNA followed by minimization makes the appropriate space between base pairs for intercalating. This approach has been applied in different sites of the DNA (base pairs 4&5, 9&10, 12&13, 15&16). Quantum calculations has been applied by using the geometric parameters and the vibrational frequencies obtained at B3LYP/6-31G+(d,p) basis using the Gaussian 09W and GaussView 05 softwares.

**ASSOCIATED CONTENT**

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXX.XXX Preliminary bioassay results, Identification of the fungal species, HPLC methods, molecular modelling images and $^{1}H$ and $^{13}C$ NMR spectra of isolated compounds.

## AUTHOR INFORMATION

Corresponding Author: * Tel: +442078481891, Email: k.miraz.rahman@kcl.ac.uk (KMR), Email: mhsohrab@yahoo.com (MHS)

Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

The authors are thankful to the authority of BCSIR for supporting the work in this paper through a fellowship to N.S.C and also for providing the lab facilities related to the work on endophytic fungi.

## REFERENCES

(1) Azevedo, J. L.; Maccheroni Jr, W.; Pereira, J. O.; de Araújo, W. L. ***Electron. J. Biotechn.*** **2000**, *3*, 15-16.

(2) Azevedo, J. L.; Macheroni, W.; Pereira, J. O. *Electron J. Biotechn.* **2001**, *3*, 1-36.

(3) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. *J.Nat. Prod.* **2004**, *67*, 257-68.

(4) Issaq, H. J.; Barr, E. W.; Wei, T.; Meyers, C.; Aszalos, A. *J. Chromatogr.* **1977**, *133*, 291-301.

(5) Andréa, M.; Nascimento, D.; Raphael, C.; Izabel, C.; Turatti, C.; Bruno, C.; Letícia, V.; Costa, L.; Cláudia, P.; Manoel, O.; Moraes, D.; Viviane, M. J.; Toledo, S.; Angela, K.; Cruz, M. T. *Rev. Bras. Farmacogn.* **2012**, *22*, 1276-1281.

(6) Takemoto, K.; Kamisuki, S.; Chia, P. T.; Kuriyama, I.; Mizushina, Y.; Sugawara, F. *J. Nat. Prod.* **2014**, *77*, 1992-6.

(7) Yang, S. X.; Gao, J. M.; Zhang, A. L.; Laatsch, H. *Bioorg. Med. Chem, Lett.* **2011**, *21*, 3905-8.

(8) Yang, S. X.; Xiao, J.; Laatsch, H.; Holstein, J. J.; Dittrich, B.; Zhang, Q.; Gao, J. M. *Tetrahedron Lett.* **2012**, *53*, 6372–6375.

(9) Yang, S. X.; Gao, J. M.; Laatsch, H.; Tian, J. M.; Pescitelli, G. *Chirality* **2012**, *24*, 621-7.

(10) Li, G.; Kusari, S.; Golz, C.; Strohmann, C.; Spiteller, M. *RSC Adv.* **2016**, *6*, 54092-54098.

(11) Kurobane, I.; Zaita, N.; Fukuda, A. *J. Antibiot.* **1986**, *39*, 205-14.

(12) Madentsev, A. G.; Arinbasarova, A.; Yu., A. *Appl. Biochem. Microbiol.* **2005**, *41*, 503-507.

(13) Robert, A. B.; James, H. T.; Stanley, N. J. *Physiol. Biochem.* **1981**, *71*, 951-954.

(14) Ericsson, D. C. B.; Ivonne, J. N. R. *Chem. Nat. Compd.* **2009**, *45*, 193-196.

(15) Schüffler, A.; Liermann, J. C.; Kolshorn, H.; Opatz, T.; Anke, H. *Z. Naturforsch. C.* **2009**, *64*, 25-31.

(16) Miljkovic, A.; Mantle, P. G.; Willium, D. J.; Rassing, B. *J. Nat. Prod.* **2001**, *64*, 1251-1253.

(17) Zerihun, T. D.; Beauty, S.; Marieka, G.; Teunis, V. R. *Nat. Prod. Res.* **2016**, *30*, 1301-4.

(18) Chowdhury, N. S.; Sohrab, M. H.; Rony , S. R.; Sharmin , S.; Begum, M. N.; Rana, M. S.; Choudhury, M. H. *Bangladesh J. Bot.* **2016**, *45*, 193-199.

(19) Alzoreky, N. S.; Nakahara, K. *Int. J. Food Microbiol.* **2003**, *80*, 223-30.

(20) Koes, D. R.; Baumgartner, M. P.; Camacho, C. J. *J. Chem. Inf. Model.* **2013**, *53*, 1893-904.

(21) Gilad, Y.; Senderowitz, H. *J. Chem. Inf. Model.* **2014**, *54*, 96-107.